

WHAT IS CLAIMED IS:

1. A composition for quantifying or detecting one or more target nucleic acid molecules in a sample, comprising at least three oligonucleotides, wherein the first oligonucleotide has a tail at the 5'-end of the gene-specific target primer which is non-complementary to the target sequence and which is identical to the 3'-end sequence of the second oligonucleotide; the second oligonucleotide is at least partially identical to the tail of the first oligonucleotide and is labeled with a fluorescent moiety; and the third oligonucleotide is a primer complementary to the 3'-end of the target.

2. A method for quantitation or detection of one or more nucleic acid molecules in a sample during nucleic acid amplification comprising:

mixing one or more nucleic acid templates with oligonucleotides of claim 1 under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of said templates, said amplified nucleic acid molecule comprising said oligonucleotides; and

detecting the presence or absence or quantifying the amount of said nucleic acid molecules by measuring the detectable labels of said oligonucleotides.

3. A method for amplifying a double-stranded nucleic acid molecule, comprising at least three oligonucleotides, wherein the first oligonucleotide has a tail at the 5'-end of the gene-specific target primer which is non-complementary to the target sequence and which is identical to the 3'-end sequence of the second oligonucleotide; the second oligonucleotide is at least partially identical to the tail of the first oligonucleotide and is labeled with a fluorescent moiety; and the third oligonucleotide is a PCR primer complementary to the 3'-end of the target.

4. A method for synthesizing or amplifying one or more nucleic acid molecules comprising:

mixing one or more nucleic acid templates or targets with one or more oligonucleotides, wherein said one or more of said oligonucleotides comprises at least one modified oligonucleotide; and

incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates or targets.

5. A method for synthesizing or amplifying one or more nucleic acid molecules, wherein the specificity of the nucleic acid synthesis or amplification is increased, comprising:

mixing one or more nucleic acid templates or targets with one or more oligonucleotides, wherein said one or more of said oligonucleotides comprises at least one hairpin structure; and

incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates or targets, wherein the synthesis or amplification has increased specificity when compared to amplification or synthesis conducted with an oligonucleotide not in a hairpin conformation.

6. A method for synthesizing or amplifying one or more nucleic acid molecules, wherein the specificity of the nucleic acid synthesis or amplification is increased, comprising:

mixing one or more nucleic acid templates or targets with one or more oligonucleotides, wherein said one or more of said oligonucleotides comprises at least one modified oligonucleotide; and

incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates or targets, wherein the synthesis or

amplification has increased specificity when compared to amplification or synthesis conducted with an unmodified oligonucleotide.

7. A method for synthesizing or amplifying one or more nucleic acid molecules, wherein the synthesis or amplification inhibits or reduces mis-priming, comprising:

mixing one or more nucleic acid templates or targets with one or more oligonucleotides, wherein said one or more of said oligonucleotides comprises at least one hairpin structure; and

incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates or targets, wherein the synthesis or amplification inhibits or reduces mis-priming when compared to amplification or synthesis conducted with an oligonucleotide not in a hairpin conformation.

8. A method for synthesizing or amplifying one or more nucleic acid molecules, wherein the synthesis or amplification inhibits or reduces mis-priming, comprising:

mixing one or more nucleic acid templates or targets with one or more oligonucleotides, wherein said one or more of said oligonucleotides comprises at least one modified oligonucleotide; and

incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates or targets, wherein the synthesis or amplification inhibits or reduces mis-priming when compared to amplification or synthesis conducted with an unmodified oligonucleotide.

9. A composition comprising one or more nucleic acid molecules and at least one oligonucleotide, wherein at least a portion of said oligonucleotide is capable of hybridizing with at least a portion of said nucleic

acid molecule and wherein said oligonucleotide comprises a modified nucleotide at or near the 3'-terminal nucleotide.

10. The composition of claim 9, wherein said modified ribonucleotide is a 2'-O- alkyl ribonucleotide.

11. The composition of claims 9 or 10, further comprising at least one component selected from the group consisting of one or more nucleotides, one or more DNA polymerases and one or more reverse transcriptases.

12. A method for amplifying a double-stranded nucleic acid molecule, comprising:

providing a first and second primer, wherein said first primer is complementary to a sequence within or at or near the 3'-termini of the first strand of said nucleic molecule and said second primer is complementary to a sequence within or at or near the 3'-termini of the second strand of said nucleic acid molecule;

hybridizing said first primer to said first strand and said second primer to said second strand in the presence of one or more of the polymerases, under conditions such that a third nucleic acid molecule complementary to all or a portion of said first strand and a fourth nucleic acid molecule complementary to all or a portion said second strand are synthesized;

denaturing said first and third strand, and said second and fourth strands; and

repeating the above steps one or more times, wherein one or more of the primers comprise a modified nucleotide at or near the 3'-terminal nucleotide.

13. The method of claim 12, wherein said modified ribonucleotide is a 2'-O- alkyl ribonucleotide.

14. A method of determining the presence of at least one nucleotide of interest at a specific position in a target nucleic acid molecule, comprising:

contacting at least one target nucleic acid molecule having said nucleotide of interest at a specific position on a target nucleic acid molecule with at least one oligonucleotide, wherein at least a portion of the oligonucleotide is capable of forming base pairs or hybridizing with at least a portion of the target nucleic acid molecule and wherein the oligonucleotide comprises a modified nucleotide at or near the 3'-terminal nucleotide; and

incubating the oligonucleotide and the target nucleic acid molecule under conditions sufficient to cause extension of the oligonucleotide when the 3'-most nucleotide of the oligonucleotide base pair with the nucleotide at the specific position of the target nucleic acid molecule, wherein the presence of or increased production of an extension product indicates the presence of the particular nucleotide at the specific position.

15. The method of claim 14, wherein said modified ribonucleotide is a 2'-O- alkyl ribonucleotide.

16. A method of determining the absence of at least one nucleotide at a specific position in a target nucleic acid molecule, comprising:

contacting at least one target nucleic acid molecule having said nucleotide of interest at a specific position on the target nucleic acid molecule with at least one oligonucleotide, wherein at least one portion of the oligonucleotide is capable of forming base pairs or hybridizing with at least a portion of the target nucleic acid molecule and wherein the oligonucleotide comprises a modified nucleotide at or near the 3'-terminal nucleotide; and

incubating the oligonucleotide and target nucleic acid molecule under conditions sufficient to inhibit or prevent extension of the oligonucleotide when the 3'-most nucleotide of the oligonucleotide does not substantially base pair with the nucleotide of the specific position of the target nucleic acid molecule, wherein the substantial reduction or no production of

an extension product indicates the absence of the particular nucleotide at the specific position.

17. The method of claim 16, wherein said modified ribonucleotide is a 2'-O- alkyl ribonucleotide.

18. A method of determining the presence or absence of a nucleotide at a specific position in a target nucleic acid molecule, comprising:

contacting at least first oligonucleotide with at least one target nucleic acid molecule under conditions sufficient to cause extension of the first oligonucleotide when the 3'-most nucleotide of the oligonucleotide base pairs with the nucleotide at the specific position of the target nucleic acid molecule, wherein said first oligonucleotide comprises a modified nucleotide at or near the 3'-terminal nucleotide;

contacting at least a second oligonucleotide with at least one target nucleic acid molecule under conditions sufficient to inhibit or prevent extension of the oligonucleotide when the 3'-most nucleotide of the oligonucleotide do not substantially base pair with the nucleotide at the specific position of the target nucleic acid molecule, wherein said second oligonucleotide comprises a modified nucleotide at or near the 3'-terminal nucleotide; and

comparing the level of extension or the amount of extension product accomplished with the first oligonucleotide compared to the second oligonucleotide.

19. The method of claim 18, wherein said modified ribonucleotide is a 2'-O- alkyl ribonucleotide.

20. A method for synthesizing or amplifying one or more nucleic acid molecules comprising:

mixing one or more nucleic acid templates or targets with one or more oligonucleotides, wherein said one or more of said oligonucleotides comprise a modified nucleotide at or near the 3'-terminal nucleotide; and

incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates or targets.

21. The method of claim 20, wherein said modified ribonucleotide is a 2'-O-alkyl ribonucleotide.

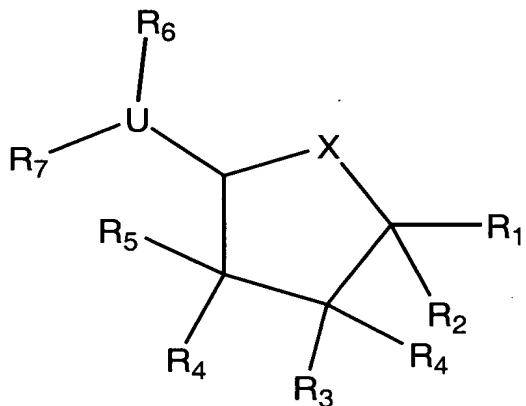
22. A method for synthesizing or amplifying one or more nucleic acid molecules, wherein the specificity of the nucleic acid synthesis or amplification is increased, comprising:

mixing one or more nucleic acid templates or targets with one or more oligonucleotides, wherein said one or more of said oligonucleotides comprises a modified ribonucleotide at or near the 3'-terminal nucleotide; and

incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates or targets, wherein the synthesis or amplification has increased specificity when compared to amplification or synthesis conducted with an oligonucleotide not modified with a modified nucleotide at or near the 3'-terminal nucleotide.

23. The method of claim 22, wherein said modified ribonucleotide is a 2'-O-alkyl ribonucleotide.

24. A nucleotide analogue having the formula:



wherein,

X is selected from the group consisting of -O-, -S-, -SO-, -SO₂-, -Se-, C(R₈R₉), -N(R₁₀R₁₁), NR₁₀, P(O₂) and P(O)-O-R₁₂;

R₁ is selected from the group consisting of nucleobases, heteroaromatic groups, heterocyclic group and aryl;

R₂ is selected from the group consisting of H, alkyl, alkoxy, alkylamino, alkylmercapto, aryl, aryloxy, carboxylic acid, carboxamide, aminoacid, hydroxyacid, peptide, sugar, hydroxy, amino and thio;

R₃ is selected from the group consisting of H, alkyl, alkoxy, alkylamino, alkylmercapto, aryl, aryloxy, carboxylic acid, carboxamide, aminoacid, hydroxyacid, peptide, sugar, hydroxy, amino and thio;

each R₄ is selected from the group consisting of hydroxy, alkoxy, amino and thio;

R₅ is selected from the group consisting of H, alkyl, alkoxy, alkylamino, alkylmercapto, aryl, aryloxy, carboxylic acid, carboxamide, aminoacid, hydroxyacid, peptide, sugar, hydroxy, amino and thio;

U is selected from the group consisting of nucleobases, heteroaromatic groups, heterocyclic group and aryl;

R₆ is R₂ (when U = CR₈, NR₁₀, N) or R₆ is absent when U = -O-, -S-, -SO-, -SO₂-, or -Se-;

R₇ is selected from the group consisting of triphosphate, diphosphate, monophosphate, phosphorotioate, oligonucleotide, nucleic acid, DNA, RNA, LNA[JW2] and PNA;

R₈ is selected from the group consisting of H, alkyl, alkyloxy, alkylamino, alkylmercapto, aryl, aryloxy, carboxylic acid, carboxamide, aminoacid, hydroxyacid, peptide, sugar, hydroxy, amino and thio;

R₉ is selected from the group consisting of H, alkyl, alkyloxy, alkylamino, alkylmercapto, aryl, aryloxy, carboxylic acid, carboxamide, aminoacid, hydroxyacid, peptide, sugar, hydroxy, amino and thio;

R₁₀, R₁₁, and R₁₂ are the same or different and are selected from the group consisting of alkyl, alkyloxy, alkylamino, alkylmercapto, aryl, aryloxy, carboxylic acid, carboxamide, aminoacid, hydroxyacid, peptide and sugar.

25. An oligonucleotide comprising one or more nucleotide analogues of claim 24.

26. A composition comprising one or more nucleic acid molecules and at least one oligonucleotide, wherein at least a portion of said oligonucleotide is capable of hybridizing with at least a portion of said nucleic acid molecule and wherein said oligonucleotide comprises one or more nucleotide analogues of claim 24.

27. A method of making a composition, comprising the steps of: providing at least one oligonucleotide; and

contacting said oligonucleotide with at least one nucleic acid molecule, wherein at least a portion of said oligonucleotide is capable of hybridizing with at least a portion of said nucleic acid molecule and wherein said oligonucleotide comprises one or more nucleotide analogues of claim 24.

28. A composition for quantifying or detecting one or more target nucleic acid molecules in a sample comprising one or more oligonucleotides and one or more target nucleic acid molecules to be detected or quantified, wherein said oligonucleotides contains one or more nucleotide analogues of claim 24.

29. A method for the quantification or detection of one or more target nucleic acid molecules in a sample comprising hybridizing one or more oligonucleotides with one or more molecules to be detected or quantified, and detecting the presence or absence and/or quantifying the amount of said target nucleic acid molecules, wherein said oligonucleotides contains one or more nucleotide analogues of claim 24.

30. A method for the quantitation or detection of one or more nucleic acid molecules in a sample during nucleic acid synthesis comprising:

mixing one or more nucleic acid templates with one or more oligonucleotides, wherein said oligonucleotides contains one or more nucleotide analogues of claim 24;

incubating said mixture under conditions sufficient to synthesize one or more nucleic acid molecules complementary to all or a portion of said templates, said synthesized nucleic acid molecule comprising said oligonucleotides; and

detecting the presence or absence or quantifying the amount of said synthesized nucleic acid molecules by measuring the amount of nucleic acid molecules synthesized in said sample.

31. A method for quantitation or detection of one or more nucleic acid molecules in a sample during nucleic acid amplification comprising:

mixing one or more nucleic acid templates with one or more oligonucleotides, wherein said oligonucleotides contains one or more nucleotide analogues of claim 24;

incubating said mixture under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of said templates, said amplified nucleic acid molecule comprising said oligonucleotides; and

detecting the presence or absence or quantifying the amount of said nucleic acid molecules by measuring the amount of nucleic acid molecules amplified in said sample.

32. The method for amplifying a double stranded nucleic acid molecule, comprising:

providing a first and second primer, wherein said first primer is complementary to a sequence within or at or near the 3'-termini of the first strand of said nucleic molecule and said second primer is complementary to a sequence within or at or near the 3'-termini of the second strand of said nucleic acid molecule;

hybridizing said first primer to said first strand and said second primer to said second strand in the presence of one or more of the polymerases, under conditions such that a third nucleic acid molecule complementary to all or a portion of said first strand and a fourth nucleic acid molecule complementary to all or a portion said second strand are synthesized;

denaturing said first and third strand, and said second and fourth strands; and

repeating the above steps one or more times, wherein one or more of the primers comprise one or more of the nucleotide analogues of claim 24.

33. A method of determining the presence of one or more particular nucleotides at a specific position or positions in a target nucleic acid molecule, comprising:

contacting at least one target nucleic acid molecule having one or more nucleotides of interest at a specific position or positions on a target nucleic

acid molecule with at least one oligonucleotide, wherein at least a portion of the oligonucleotide is capable of forming base pairs or hybridizing with at least a portion of the target nucleic acid molecule and wherein the oligonucleotide comprises one or more nucleotides of claim 24; and

incubating the oligonucleotide and the target nucleic acid molecule under conditions sufficient to cause extension of the oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide base pair with the nucleotide or nucleotides at the specific position or positions of the target nucleic acid molecule, wherein the production of an extension product indicates the presence of the particular nucleotide at the specific position.

34. A method of determining the absence of one or more particular nucleotides at a specific position or positions in a target nucleic acid molecule, comprising:

contacting at least one target nucleic acid molecule having one or more nucleotides of interest at a specific position or positions on the target nucleic acid molecule with at least one oligonucleotide, wherein at least one portion of the oligonucleotide is capable of forming base pairs or hybridizing with at least a portion of the target nucleic acid molecule and wherein the oligonucleotide comprises one or more nucleotides of claim 24; and

incubating the oligonucleotide and target nucleic acid molecule under conditions sufficient to inhibit or prevent extension of the oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide does not substantially base pair with the nucleotide or nucleotides of the specific position or positions of the target nucleic acid molecule, wherein the lack of or reduced production of an extension product indicates the absence of the particular nucleotide at the specific position.

35. A method of determining the presence or absence of one or more particular nucleotides at a specific position or positions in a target nucleic acid molecule, comprising:

contacting at least first oligonucleotide with at least one target nucleic acid molecule under conditions sufficient to cause extension of the first oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide base pairs with the nucleotide or nucleotides at the specific position or positions of the target nucleic acid molecule;

contacting at least a second oligonucleotide with at least one target nucleic acid molecule under conditions sufficient to inhibit or prevent extension of the oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide do not substantially base pair with the nucleotide or nucleotides at the specific position or positions of the target nucleic acid molecule; and

comparing the level of extension or the amount of extension product accomplished with the first oligonucleotide compared to the second oligonucleotide, wherein said first and/or second oligonucleotide comprises one or more nucleotides of claim 24.

36. A method of determining the presence or absence of at least one particular nucleotide of interest at a specific position in a target nucleic acid molecule, comprising:

providing at least one target nucleic acid molecule having said nucleotide of interest at a specific position;

contacting said target nucleic acid molecule with at least one oligonucleotide, wherein at least a portion of the oligonucleotide is capable of forming base pairs or hybridizing with at least a portion of the nucleic acid molecule and wherein the oligonucleotide comprises at least one nucleotide of claim 24;

contacting the oligonucleotide and the target nucleic acid molecule with a polymerase less able to extend the oligonucleotide when the 3'-most nucleotide of the oligonucleotide does not base pair with the target nucleic acid and more able to extend the oligonucleotide when the 3'-most nucleotide of the oligonucleotide base pairs with the target nucleic acid molecule; and
measuring the level of extension of the oligonucleotide.

37. A method for synthesizing or amplifying one or more nucleic acid molecules comprising:

mixing one or more nucleic acid templates or targets with one or more oligonucleotides, wherein said oligonucleotides comprises one or more nucleotide analogues of claim 24 ; and

incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates or targets.

38. A kit for use in synthesis of a nucleic acid molecule, said kit comprising one or more oligonucleotides comprising one or more of the nucleotide analogues of claim 24.

39. A kit for use in amplification of a nucleic acid molecule, said kit comprising one or more oligonucleotides comprising one or more of the nucleotide analogues of claim 24.

40. A method of detecting a single nucleotide polymorphism comprising the steps of:

contacting at least a first oligonucleotide with at least one target nucleic acid molecule under conditions sufficient to cause extension of the first oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide base pairs with the nucleotide or nucleotides at the specific position or positions of the target nucleic acid molecule;

contacting at least a second oligonucleotide with at least one target nucleic acid molecule under conditions sufficient to inhibit or prevent extension of the oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide do not substantially base pair with the nucleotide or nucleotides at the specific position or positions of the target nucleic acid molecule; and

comparing the level of extension or the amount of extension product accomplished with the first oligonucleotide compared to the second oligonucleotide, wherein said first and/or second oligonucleotide comprises one or more nucleotides of claim 24.

41. A kit for the detection or measurement of nucleic acid synthesis or amplification products comprising one or more oligonucleotides comprising one or more nucleotide analogues of claim 24.

42. An oligonucleotide comprising:
a cytosine or guanine or analog of said cytosine or guanine thereof at the 3'-termini, and
one or more detectable labels on at least the second, third, fourth, fifth or sixth base from the 3'-termini.

43. The oligonucleotide of claim 42, further comprising one or more guanines within three nucleotides flanking the labeled nucleotide on the 5'-end.

44. The oligonucleotide of claim 42, capable of forming a hairpin.

45. The oligonucleotide of claim 42, having a single detectable label.

46. The oligonucleotide of claim 42, wherein said base is a thymidine.

47. The oligonucleotide of claim 42, wherein said detectable labels are selected from the group consisting of fluorescent labels, chemiluminescent labels and bioluminescent labels.

48. The oligonucleotide of claim 47, wherein said fluorescent labels are selected from the group consisting of FAM, TAMRA, JOE, Rhodamine, BODIPY, R6G, ROX, and EDANS.

49. The oligonucleotide of claim 44, wherein said hairpin is blunt-ended.

50. The oligonucleotide of claim 44, wherein said hairpin comprises an overhanging guanine.

51. An oligonucleotide comprising:
an adenine or guanine at the 3'-termini,
an overhanging guanine at the 5'-termini, and
one or more detectable labels on at least the second, third, fourth, fifth or sixth base from a 3'-termini.

52. The oligonucleotide of claim 51, further comprising one or more guanines within three nucleotides flanking the labeled nucleotide on the 5'-end.

53. The oligonucleotide of claim 51, capable of forming a hairpin.

54. The oligonucleotide of claim 51, having a single detectable label.

55. The oligonucleotide of claim 51, wherein said base is a thymidine.

56. The oligonucleotide of claim 51, wherein said detectable labels are selected from the group consisting of fluorescent labels, chemiluminescent labels and bioluminescent labels.

57. The oligonucleotide of claim 56, wherein said fluorescent labels are selected from the group consisting of FAM, TAMRA, JOE, Rhodamine, BODIPY, R6G, ROX, and EDANS.

58. A composition comprising one or more nucleic acid molecules and at least one oligonucleotide of claims 42 or 51, wherein a portion of said oligonucleotide is capable of hybridizing with at least a portion of said nucleic acid molecule.

59. The composition of claim 58, further comprising at least one component selected from the group consisting of one or more nucleotides, one or more DNA polymerases and one or more reverse transcriptases.

60. A method of making a composition, comprising the steps of: providing at least one oligonucleotide; and

contacting said oligonucleotide with at least one nucleic acid molecule, wherein at least a portion of said oligonucleotide is capable of hybridizing with at least a portion of said nucleic acid molecule and wherein said oligonucleotide comprises said oligonucleotide of claims 42 or 51.

61. A composition for quantifying or detecting one or more target nucleic acid molecules in a sample comprising one or more oligonucleotides

and one or more target nucleic acid molecules to be detected or quantified, wherein said oligonucleotides contain said oligonucleotide of claims 42 or 51.

62. A method for the quantification or detection of one or more target nucleic acid molecules in a sample comprising hybridizing one or more oligonucleotides with one or more molecules to be detected or quantified, and detecting the presence or absence and/or quantifying the amount of said target nucleic acid molecules, wherein said oligonucleotides contain an oligonucleotide of claims 42 or 51.

63. A method for the quantitation or detection of one or more nucleic acid molecules in a sample during nucleic acid synthesis comprising:

mixing one or more nucleic acid templates with one or more oligonucleotides, wherein said oligonucleotides contain an oligonucleotide of claims 41 or 51;

incubating said mixture under conditions sufficient to synthesize one or more nucleic acid molecules complementary to all or a portion of said templates, said synthesized nucleic acid molecule comprising said oligonucleotides; and

detecting the presence or absence or quantifying the amount of said synthesized nucleic acid molecules by measuring the amount of nucleic acid molecules synthesized in said sample.

64. The method for amplifying a double stranded nucleic acid molecule, comprising:

providing a first and second primer, wherein said first primer is complementary to a sequence within or at or near the 3'-termini of the first strand of said nucleic molecule and said second primer is complementary to a sequence within or at or near the 3'-termini of the second strand of said nucleic acid molecule;

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repeating the above steps one or more times, wherein one or more of the primers comprise an oligonucleotide of claims 42 or 51.

contacting at least one target nucleic acid molecule having one or more nucleotides of interest at a specific position or positions on a target nucleic acid molecule with at least one oligonucleotide, wherein at least a portion of the oligonucleotide is capable of forming base pairs or hybridizing with at least a portion of the target nucleic acid molecule and wherein the oligonucleotide comprises an oligonucleotide of claims 41 or 51; and

incubating the oligonucleotide and the target nucleic acid molecule under conditions sufficient to cause extension of the oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide base pair with the nucleotide or nucleotides at the specific position or positions of the target nucleic acid molecule, wherein the production of an extension product indicates the presence of the particular nucleotide at the specific position.

contacting at least one target nucleic acid molecule having one or more nucleotides of interest at a specific position or positions on the target

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nucleic acid molecule with at least one oligonucleotide, wherein at least one portion of the oligonucleotide is capable of forming base pairs or hybridizing with at least a portion of the target nucleic acid molecule and wherein the oligonucleotide comprises an oligonucleotide of claims 41 or 51; and

incubating the oligonucleotide and target nucleic acid molecule under conditions sufficient to inhibit or prevent extension of the oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide does not substantially base pair with the nucleotide or nucleotides of the specific position or positions of the target nucleic acid molecule, wherein the lack of or reduced production of an extension product indicates the absence of the particular nucleotide at the specific position.

67. A method of determining the presence or absence of one or more particular nucleotides at a specific position or positions in a target nucleic acid molecule, comprising:

contacting at least first oligonucleotide with at least one target nucleic acid molecule under conditions sufficient to cause extension of the first oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide base pairs with the nucleotide or nucleotides at the specific position or positions of the target nucleic acid molecule;

contacting at least a second oligonucleotide with at least one target nucleic acid molecule under conditions sufficient to inhibit or prevent extension of the oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide do not substantially base pair with the nucleotide or nucleotides at the specific position or positions of the target nucleic acid molecule; and

comparing the level of extension or the amount of extension product accomplished with the first oligonucleotide compared to the second oligonucleotide, wherein said first and/or second oligonucleotide comprises an oligonucleotide of claims 41 or 51.

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68. A method of determining the presence or absence of at least one particular nucleotide of interest at a specific position in a target nucleic acid molecule, comprising:

providing at least one target nucleic acid molecule having said nucleotide of interest at a specific position;

contacting said target nucleic acid molecule with at least one oligonucleotide, wherein at least a portion of the oligonucleotide is capable of forming base pairs or hybridizing with at least a portion of the nucleic acid molecule and wherein the oligonucleotide comprises at least one oligonucleotide of claims 42 or 51;

contacting the oligonucleotide and the target nucleic acid molecule with a polymerase less able to extend the oligonucleotide when the 3'-most nucleotide of the oligonucleotide does not base pair with the target nucleic acid and more able to extend the oligonucleotide when the 3'-most nucleotide of the oligonucleotide base pairs with the target nucleic acid molecule; and

measuring the level of extension of the oligonucleotide.

69. A method for synthesizing or amplifying one or more nucleic acid molecules comprising:

mixing one or more nucleic acid templates or targets with one or more oligonucleotides, wherein said oligonucleotides comprise an oligonucleotide of claims 42 or 51; and

incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates or targets.

70. A method of detecting a single nucleotide polymorphism comprising the steps of:

contacting at least a first oligonucleotide with at least one target nucleic acid molecule under conditions sufficient to cause extension of the first oligonucleotide when the 3'-most nucleotide or nucleotides of the

oligonucleotide base pairs with the nucleotide or nucleotides at the specific position or positions of the target nucleic acid molecule;

contacting at least a second oligonucleotide with at least one target nucleic acid molecule under conditions sufficient to inhibit or prevent extension of the oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide do not substantially base pair with the nucleotide or nucleotides at the specific position or positions of the target nucleic acid molecule; and

comparing the level of extension or the amount of extension product accomplished with the first oligonucleotide compared to the second oligonucleotide, wherein said first and/or second oligonucleotide comprises an oligonucleotide of claims 42 or 51.

71. A method for quantitation or detection of one or more nucleic acid molecules in a sample during nucleic acid amplification comprising:

mixing one or more nucleic acid templates with an oligonucleotide of claims 42 or 51 under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of said templates, said amplified nucleic acid molecule comprising said oligonucleotides; and

detecting the presence or absence or quantifying the amount of said nucleic acid molecules by measuring the detectable labels of said oligonucleotides.

72. A kit for use in the synthesis of a nucleic acid molecule, said kit comprising one or more oligonucleotides of claims 42 or 51.

73. A kit for use in the amplification of a nucleic acid molecule, said kit comprising one or more oligonucleotides of claims 42 or 51.

1. The first part of the paper discusses the importance of the research and the objectives of the study.